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# Low-frequency ultrasound as a tool for quality control of soft-bodied raw ewe's milk cheeses

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ABSTRACT

The relationship between the quality attributes and the ultrasound parameters of Protected Designation of Origin (PDO) soft-bodied cheeses from "Torta del Casar" and "Queso de la Serena" was studied. Cheese samples were taken from six different dairy industries (three for each PDO) in two different seasons. Ultrasound parameters related to velocity, attenuation and frequency were calculated and correlated with the physico-chemical, microbiological, rheological and sensorial properties of the cheeses. Several ultrasonic parameters showed a high correlation with changes in the physico-chemical, textural and sensorial properties of this product, which were partially associated with microbial activity. Among these, stand out the negative correlation of the sensorial descriptor "firmness" with bulk modulus ($\kappa$). The predictive models achieved by the multivariate linear regression method (MLR) showed the highest correlation coefficients for firmness by texture compression analysis (TCA) and the sensory descriptor "intensity", including as the explanatory variables for both models, fast Fourier transform (FFT) frequencies and attenuation parameters. This work highlights the on-line capability of ultrasound for non-destructive quality assessment of this traditional and heterogeneous soft cheese based on the predictive potential of several secondary ultrasonic parameters.

## 1. Introduction

Protected Designation of Origin (PDO) Spanish and Portuguese soft-bodied cheeses are highly appreciated by consumers. Although their manufacturing and ripening processes vary, they are always made from raw ewe's milk without any added starter culture and an aqueous infusion of dried flowers of *Cynara cardunculus* L. as rennet, which gives them unique sensory properties. In the region of Extremadura (Spain) are located two of the most appreciated Protected Designations of Origin cheeses of this type: "Torta del Casar" and "Queso de la Serena". However, the final characteristics of these traditional chesses presents great variability depending on the milk-clotting activity of the vegetable coagulant and the indigenous microbiota present in raw ewe's milk (Ordiales et al., 2013 a, b; Tabla et al., 2016). The quality of a vegetable coagulant depends on the diversity of the natural cardoon populations harvested, the flower ripening stages and their drying conditions (Ordiales et al., 2012); these factors influence both the physico-chemical parameters in the initial stages of cheese processing and the texture and sensorial properties of the final product (Ordiales et al., 2014). Likewise, the absence of a starter culture can lead to uncontrolled growth of undesirable microorganisms that adversely influence the texture and taste of this type of cheese (Ordiales, Martín, et al., 2013; Tabla et al., 2016), resulting in consumer dissatisfaction. Thus, industries and distributors have a great interest in the quality control of soft-bodied cheeses such as "Torta del Casar" and "Queso de la Serena" by fast, reliable and non-destructive techniques mainly for their rheological characterization and classification.

Ultrasound-based technologies have been proposed as a quality-

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**Table 1**
Ultrasonic parameters related to velocity.

| Parameters | Equations | |
|---|---|---|
| $UPV_S$[a] | $d/TOF_S$ | *d*: distance between transducers<br>$TOF_S$: shear wave time of flight |
| $UPV_L$[b] | $d/TOF_L$ | $TOF_L$: longitudinal wave time of flight |
| Young's modulus ($E$): | $E = \frac{\rho \cdot UPV_S^2 \cdot (3UPV_L^2 - 4UPV_S^2)}{UPV_L^2 - UPV_S^2}$ | $\rho$: specific weight |
| Shear modulus ($\mu$): | $\mu = \rho \cdot UVP_S^2$ | |
| Bulk modulus ($k$): | $k = \frac{\rho \cdot (3UPV_L^2 - 4UPV_S^2)}{3}$ | |
| Lamé's constant ($\lambda$): | $\lambda = \rho \cdot (UPV_L^2 - 2UPV_S^2)$ | |
| Poisson's ratio ($\nu$): | $\nu = \frac{(UPV_L^2 - 2UPV_S^2)}{2 \cdot (UPV_L^2 - UPV_S^2)}$ | |

[a] Ultrasonic shear velocity.
[b] Ultrasonic longitudinal velocity.

control tool to determine the optimum cut time during cheese manufacturing (Jiménez et al., 2017; Koc & Ozer, 2008) and to screen process anomalies in-line (Crespo et al., 2020; Telis-Romero, Váquiro, Bon, & Benedito, 2011). Contact or non-contact low-frequency ultrasound has also been used to evaluate the texture of different types of cheeses, such as Manchego, Mahon and Cheddar (Benedito, Carcel, Sanjuan, & Mulet, 2000; Benedito, Carcel, Gonzalez, & Mulet, 2002; Benedito, Simal, Clemente, & Mulet, 2006; Cho, Irudayaraj, & Omata, 2001; Cho & Irudayaraj, 2003; Nassar et al., 2010). However, most of the studies carried out to detect texture defects in cheeses and other foods have been achieved on the basis of changes in ultrasonic velocity and, exceptionally, the attenuation when the echoes are clearly perceived on the A-scan. Nonetheless, other secondary ultrasound parameters, such as the frequency components extracted from fast Fourier transform (FFT) and attenuation, less commonly used in the literature, have shown a high characterization and discrimination capacity, being successfully applied to different food matrices (González-Mohino, Jiménez, Paniagua, Perez-Palacios, & Rufo 2019 a; González-Mohino, Jiménez, Rufo, et al., 2019; Jiménez et al., 2017). The physical properties of cooked pork loin associated with the relevant sensory attributes have been significantly ($p < 0.05$) correlated with ultrasound parameters, especially with those related to the FFT and attenuation (González-Mohino et al., 2021). Likewise, in the discrimination of defective cheeses during the soft cheese-manufacturing and ripening processes by ultrasound, attenuation and ultrasonic parameters related to the FFT play a relevant role (Crespo et al., 2020).

The objective of this work was to assess the feasibility of ultrasound as a tool for quality control of PDO soft-bodied raw ewe's milk cheeses, relating the ultrasonic parameters with the physico-chemical, microbial, textural and sensorial characteristics of the cheeses. In addition, ultrasonic parameters related to velocity, FFT and attenuation were used to construct regression models to predict the sensory attributes of chesses.

## 2. Materials and methods

### 2.1. Cheese sampling

"Torta" cheese samples with approximately 700 gr and dimension of 11 cm (diameter) and 5.5 cm (height) were collected at the end of the ripening process (60 days) from six different dairy industries, three from "Torta del Casar" PDO (Casar batch) and three from "Queso de la Serena" PDO (Serena batch), in two different seasons, winter and spring. Six cheese per industry in each season were collected at random and transported under refrigerated conditions (<5 °C) for ultrasonic inspection. Subsequently, all other assessments were conducted in less than 72 h. All cheese samples were stored under refrigerated conditions (<5 °C) until analysis. Each determination was performed in duplicate for every cheese sample (n = 72).

### 2.2. Ultrasonic measurements

The cheeses, stored at 5 °C, were immediately inspected ultrasonically using contact techniques in through-transmission (T-T) mode following the experimental set-up and methodology previously described by Crespo et al. (2020) in "Torta" cheese. In order to check the dependence of the propagation velocity with the frequency of the ultrasonic signal used, it was decided to inspect the samples with two resonance frequencies different. For this issue, two different pairs of shear wave transducers of 250 and 500 kHz frequencies, models V150-RB and V151-RB, respectively, were used (Olympus Panametrics NDT, Waltham, MA, USA). For emission and reception of the ultrasonic signals with the V150-RB and V151-RB transducers, the pulser–receiver models 5058 PR and 5077 PR (Olympus Panametrics NDT) were used, respectively. For the acquisition and digitisation of the signals, the pulser–receiver units were connected to an InfiniiVision DSO-X 3032A oscilloscope from KEYSIGHT (350 MHz bandwidth, 4 GSa/s sample rate and 10,000-point record length), which stored the data (.csv format) of the displayed signals for subsequent processing and analysis.

The parameters related to the velocity of the ultrasound are shown in Table 1. For each piece of cheese, constants were calculated according to the expressions established by the American Society for Testing and Materials (ASTM International, 2008) in protocol ASTM D2845-08. Fast Fourier transform (FFT) frequencies corresponding to the 25th, 50th, 75th and 99th ($FFT_{25}$, $FFT_{50}$, $FFT_{75}$ and $FFT_{99}$, respectively) percentiles of the received signals in the cumulative frequency periodograms of the FFT and the highest amplitude frequency, as well as the attenuation quantified in terms of the time taken to receive the energy (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% and 90% of the total energy received, denoted as AT 10, AT 20, …, AT 90, respectively), expressed as a multiple of the TOF (n•TOF), were also recovered (Cerrillo, Jiménez, Rufo, ). These parameters were determined along the centre of opposite faces of the specimens for each of the selected inspection frequencies.

### 2.3. Microbial counts

The cheeses were cut aseptically and a core sample of approximately 10 g was placed under sterile conditions in a stomacher bag and diluted 10 times with sterile peptone water. (Condalab, Madrid, Spain). After homogenisation for 120 s in a stomacher instrument (Lab-Blender 400 Seward Lab., London, England), serial 10-fold dilutions were performed with sterile peptone water, and aliquots of 0.1 mL from each dilution were inoculated onto agar plates. Plate count agar (PCA, Condalab; 30 °C/24 h) was used for enumeration of aerobic mesophilic bacteria (AMB); de Man, Rogosa and Sharpe (MRS; Condalab) agar acidified to pH 5.6 with acetic acid (10%) for mesophilic (MLAB) and thermophilic lactic acid bacteria (TLAB) after incubation at 30 °C and 42 °C for 48 h, respectively; Slanetz and Bartley (SB, Condalab; 37 °C/48 h) for enterococci; Baird-Parker agar (BP, Condalab; 37 °C/48 h) supplemented with potassium tellurite and egg yolk emulsion (Condalab) for gram-positive catalase-positive cocci (GPCPC); Violet Red Bile Glucose agar (VRBG, Condalab; 30 °C/24 h) for enterobacteria; Violet Red Bile Lactose agar (VRBA, Condalab; 37 °C/24 h) for coliforms; chromogenic tryptone bile X-glucuronide agar (TBX, Condalab; 44 °C/24 h) for *Escherichia coli* and Potato Dextrose agar (PDA, Condalab; 25 °C/4 days) acidified to pH 3.5 with a sterilised solution of tartaric acid (10%) for yeast and moulds.

### 2.4. Physico-chemical analysis

Dry matter, moisture, fat, protein and NaCl contents were

**Table 2**
Descriptive statistics of ultrasonic parameters measured in the cheese batches.

| Ultrasonic parameter | Transducer V150-RB | | | | | Transducer V151-RB | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD[a] | %RSD[b] | Min[c] | Max[c] | Mean | SD | %RSD | Min | Max |
| *Velocity-related parameters* | | | | | | | | | | |
| $UPV_L$ (m/s) | 1,571 | 95 | 6.06 | 1,101 | 1,731 | 1,611 | 65 | 4.02 | 1,316 | 1,703 |
| $UPV_S$ (m/s) | 789 | 74 | 9.39 | 561 | 940 | 834 | 74 | 8.88 | 574 | 1,018 |
| $UPV_S/UPV_L$ | 0.502 | 0.032 | 6.36 | 0.430 | 0.610 | 0.518 | 0.039 | 7.61 | 0.420 | 0.610 |
| Young's modulus ($E$) ($N/m^2$) | 1.63E+09 | 3.09E+08 | 19.00 | 7.82E+08 | 2.36E+09 | 1.79E+09 | 3.09E+08 | 17.27 | 8.96E+08 | 2.54E+09 |
| Poisson's ratio ($\nu$) | 0.330 | 0.030 | 9.22 | 0.200 | 0.390 | 0.313 | 0.041 | 12.98 | 0.200 | 0.390 |
| Bulk modulus ($\kappa$) ($N/m^2$) | 1.60E+09 | 2.35E+08 | 14.67 | 6.60E+08 | 2.03E+09 | 1.63E+09 | 2.18E+08 | 13.41 | 1.00E+09 | 1.97E+09 |
| Shear modulus ($\mu$) ($N/m^2$) | 6.14E+08 | 1.27E+08 | 20.71 | 3.00E+08 | 9.17E+08 | 6.84E+08 | 1.34E+08 | 19.62 | 3.22E+08 | 1.06E+09 |
| Lamé's constant ($\lambda$) ($N/m^2$) | 1.19E+09 | 2.13E+08 | 17.85 | 4.60E+08 | 1.67E+09 | 1.17E+09 | 2.32E+08 | 19.80 | 5.10E+08 | 1.57E+09 |
| *Frequencies* | | | | | | | | | | |
| HAF[d] (Hz) | 274,666 | 46,009 | 16.75 | 125,219 | 420,787 | 341,683 | 137,617 | 40.28 | 164,703 | 604,222 |
| $FFT_{25}$ (Hz) | 247,145 | 33,041 | 13.37 | 160,815 | 321,881 | 288,721 | 78,570 | 27.21 | 164,866 | 486,103 |
| $FFT_{50}$ (Hz) | 283,882 | 27,224 | 9.59 | 226,368 | 357,645 | 388,151 | 94,559 | 24.36 | 224,336 | 580,465 |
| $FFT_{75}$ (Hz) | 323,080 | 27,019 | 8.36 | 251,319 | 420,787 | 500,362 | 83,706 | 16.73 | 290,768 | 691,910 |
| $FFT_{99}$ (Hz) | 514,269 | 60,324 | 11.73 | 380,305 | 630,755 | 754,806 | 64,038 | 8.48 | 603,658 | 913,680 |
| *Attenuations* | | | | | | | | | | |
| AT 10 | 1.41 | 0.31 | 21.91 | 1.06 | 2.19 | 1.29 | 0.23 | 17.97 | 1.04 | 2.14 |
| AT 20 | 1.58 | 0.41 | 25.88 | 1.07 | 2.77 | 1.43 | 0.33 | 23.03 | 1.05 | 2.50 |
| AT 30 | 1.74 | 0.48 | 27.89 | 1.10 | 3.07 | 1.56 | 0.40 | 25.59 | 1.05 | 2.79 |
| AT 40 | 1.88 | 0.55 | 29.26 | 1.11 | 3.21 | 1.70 | 0.48 | 28.48 | 1.07 | 3.11 |
| AT 50 | 2.06 | 0.62 | 30.20 | 1.12 | 3.36 | 1.87 | 0.56 | 30.13 | 1.08 | 3.37 |
| AT 60 | 2.27 | 0.71 | 31.24 | 1.16 | 3.76 | 2.09 | 0.64 | 30.74 | 1.11 | 3.75 |
| AT 70 | 2.56 | 0.86 | 33.48 | 1.19 | 4.28 | 2.36 | 0.76 | 32.17 | 1.14 | 4.24 |
| AT 80 | 2.88 | 0.98 | 34.06 | 1.24 | 4.88 | 2.78 | 0.93 | 33.36 | 1.21 | 5.30 |
| AT 90 | 3.64 | 1.21 | 33.12 | 1.30 | 6.14 | 3.51 | 1.12 | 31.90 | 1.51 | 6.59 |

[a] SD: standard deviation.
[b] %RSD: relative standard deviation expressed as a percentage.
[c] Min (minimum); Max (maximum).
[d] HAF: Highest amplitude frequency.

determined according to standard methods: dry matter and moisture (International Organization for Standardization, 2004), fat (International Organization for Standardization, 2008), protein (International Organization for Standardization, 2014) and NaCl (International Organization for Standardization, 2006a). The acidity (lipolysis index) was measured by the titration method as described by De Filippis, Genovese, Ferranti, Gilbert, and Ercolini (2016).

### 2.5. Texture assessment

To evaluate the rheological characteristics of cheese samples, two different assays were conducted using a TA.XT2i Texture Analyzer (Stable Micro Systems, Godalming, UK). In both assays, the operational parameters of the instrument were as follows: pre-test speed, 3 mm/s; test speed, 2.00 mm/s; post-test speed, 10.00 mm/s; distance, 10.0 mm and trigger force, 25 g. Firstly, a texture compression analysis (TCA) was performed by fitting the analyzer instrument with a 30-kg load cell (Stable Micro Systems, Godalming, UK) and spherical stainless-steel probe (P/1S). Cheese sample at 5 °C were positioned under the probe and measured on both sides (top and bottom) in the centre. In total, two measurements per cheese were carried out. The values for firmness (g) and stickiness (g) of the cheese were calculated using the Exponent software version 5.0.4.0 (Stable Micro System).

In addition, a texture spreadability analysis (TSA) was performed using an HDP/SR spreadability probe and instrument equipped with a 5-kg load cell (Stable Micro Systems). The cheeses were tempered at 20 °C, and a sample from the core of the cheese was packed into the lower cone with a spatula and measured at 20 °C. In the resulting graphs, firmness (g) and work of shear (g·s) were measured as the maximum peak and the area under the first curve using Exponent software version 5.0.4.0 (Stable Micro System), while the maximum negative peak was used to calculate the stickiness (g) of the sample, and the maximum negative area the work of adhesion (g·s).

### 2.6. Sensory evaluation

To evaluate the sensory quality of the cheese, a descriptive analysis was performed according to international standard methods by a panel of 20 panellists. Members of the panel were previously selected and trained under International Organization for Standardization standards (International Organization for Standardization, 2006b) with samples of commercial "Torta" cheese. All sessions were conducted in a sensory panel room conditioned at 20–22 °C and 60%–70% relative humidity in cabinets equipped with white light (6000 °K). Before the analysis, the cheeses were equilibrated at sensory room temperature. A portion of approximately of 10 g of cheese paste and 4 $cm^2$ of rind per sample was presented individually in a plastic dish for tasting. Samples were 3-digit coded, and presented in random order. Between samples, mineral water and bread were supplied for cleansing the palate. In each session, only three different cheese samples were evaluated. Twelve sensory attributes related to the colour of the paste and rind (from light yellow to dark yellow), texture (firmness and creaminess), taste (salty, acidic, bitter, spicy and astringent), pungency sensation and flavour (intensity, persistence and rancidity) were assessed quantitatively using a structured numerical scale scored from 0 (low intensity) to 9 (high intensity). Two panel replicates were carried out on each sample. The response to each attribute for each panellist was determined as the mean value of his or her responses. In addition to this descriptive evaluation, a panel of 50 untrained consumers evaluated the samples for overall acceptability.

### 2.7. Statistical data analysis

Data were analysed statistically using SPSS Statistics, Version 21.0 for Windows (Armonk, NY: IBM, Inc.). Descriptive statistics of the ultrasonic, microbial, physico–chemical, textural and sensory determinations between the two PDO samples was studied by one-way analysis of variance (ANOVA) and separated by Tukey's honestly significant differences test ($p \leq 0.05$). The relationship between ultrasonic parameters and microbial, physico-chemical, texture and sensory data of

**Table 3**
Microbial, physico-chemical and textural parameters of soft cheeses.

| Parameters[a] | Casar batch | | | | | Serena batch | | | | | P value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | %RSD | Max | Min | Mean | SD | %RSD | Max | Min | |
| *Microbial parameters (log CFU $g^{-1}$)*[c] | | | | | | | | | | | |
| AMB | 8.6 | 0.4 | 4.5 | 9.0 | 8.0 | 8.8 | 0.2 | 2.2 | 9.1 | 8.6 | 0.334 |
| MLAB | 8.3 | 0.4 | 4.7 | 8.7 | 7.8 | 8.7 | 0.2 | 2.8 | 8.9 | 8.4 | 0.067 |
| TLAB | 6.1 | 0.8 | 13.1 | 6.8 | 4.7 | 5.6 | 1.4 | 24.2 | 7.4 | 4.0 | 0.505 |
| GPCPC | 5.1 | 0.5 | 9.9 | 5.6 | 4.4 | 4.9 | 1.1 | 23.6 | 6.4 | 3.5 | 0.661 |
| Enterococci | 6.4 | 0.9 | 13.7 | 6.9 | 4.7 | 5.6 | 0.7 | 12.4 | 6.5 | 4.9 | 0.090 |
| Enterobacteriaceae | 5.8 | 1.6 | 28.5 | 7.6 | 3.6 | 5.9 | 1.0 | 16.5 | 7.0 | 4.7 | 0.842 |
| Total coliforms | 5.5 | 1.9 | 34.1 | 7.4 | 3.0 | 5.6 | 1.0 | 17.4 | 6.7 | 4.5 | 0.826 |
| Yeasts | 3.3 | 0.7 | 19.9 | 4.1 | 2.6 | 4.0 | 0.4 | 10.7 | 4.3 | 3.1 | 0.068 |
| Moulds | 0.06 | 0.14 | 245 | 0.33 | 0.00 | 0.07 | 0.16 | 245 | 0.40 | 0.00 | 0.901 |
| *Physico-chemical parameters* | | | | | | | | | | | |
| Moisture (%) | 46.7 | 3.5 | 7.6 | 50.4 | 40.3 | 45.8 | 2.1 | 4.6 | 49.3 | 43.6 | 0.627 |
| Total protein (%) | 21.4 | 0.9 | 4.3 | 22.5 | 20.2 | 21.1 | 0.8 | 3.9 | 22.6 | 20.4 | 0.509 |
| Total protein DM[b] (%) | 40.4 | 2.5 | 6.2 | 42.9 | 37.3 | 39.0 | 2.0 | 5.1 | 42.2 | 37.1 | 0.323 |
| Fat (%) | 29.4 | 2.9 | 9.9 | 32.8 | 25.6 | 29.1 | 3.2 | 10.9 | 33.8 | 25.3 | 0.860 |
| Fat DM (%) | 55.1 | 4.2 | 7.6 | 58.8 | 47.8 | 53.6 | 4.4 | 8.3 | 60.2 | 47.2 | 0.550 |
| Salt (%) | 2.3 | 1.0 | 43.6 | 4.4 | 1.5 | 2.1 | 0.7 | 33.4 | 3.5 | 1.5 | 0.715 |
| pH | 5.8 | 0.3 | 5.0 | 6.1 | 5.4 | 5.4 | 0.2 | 3.7 | 5.6 | 5.1 | 0.025 |
| Acidity (mg KOH $g^{-1}$) | 12.8 | 5.1 | 39.6 | 18.1 | 5.6 | 8.1 | 3.2 | 39.8 | 12.1 | 3.2 | 0.088 |
| *Texture compression analysis (TCA)* | | | | | | | | | | | |
| Firmness (g) | 3796 | 3120 | 82.2 | 9986 | 1670 | 3412 | 2209 | 65 | 7349 | 1121 | 0.811 |
| Stickiness (g) | −39.8 | 39.4 | −99.0 | −4.8 | −113 | −28.7 | 25.6 | −89.5 | −3.7 | −59.0 | 0.574 |
| *Texture spreadability analysis (TSA)* | | | | | | | | | | | |
| Firmness (g) | 723 | 635 | 87.8 | 1593 | 73 | 805.8 | 317.3 | 39.4 | 1288 | 363 | 0.782 |
| Work of Shear (g•s) | 0.028 | 0.023 | 81.7 | 0.070 | 0.008 | 0.022 | 0.011 | 52.0 | 0.041 | 0.008 | 0.549 |
| Stickiness (g) | −449 | 395 | −87.9 | −50 | −987 | −606 | 318 | −53 | −193 | −995 | 0.467 |
| Work of adhesion (g•s) | −166 | 108 | −65.0 | −56 | −320 | −230 | 112 | −49 | −72 | −383 | 0.337 |

[a] SD: standard deviation; %RSD percent relative standard deviation.
[b] DM: Dry mater.
[c] AMB: aerobic mesophilic bacteria; MLAB: mesophilic lactic acid bacteria; TLAB: thermophilic lactic acid bacteria; GCCPC: Gram-positive catalase-positive cocci.

**Table 4**
Sensory descriptive attributes of soft cheeses and their correlation with overall acceptance.

| Sensory attributes | Casar | | | | | Serena | | | | | P value[1] | Overall Accep.[2] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | %RSD | Max | Min | Mean | SD | %RSD | Max | Min | | Coef | Sig. |
| Appearance (rind) | 7.63 | 0.49 | 6.5 | 8.2 | 7.0 | 5.34 | 1.84 | 34.4 | 8.0 | 3.2 | **0.014** | 0.612 | ** |
| Appearance (paste) | 4.43 | 0.60 | 13.4 | 5.2 | 3.7 | 4.00 | 0.98 | 24.5 | 5.6 | 2.6 | 0.378 | 0.214 | |
| Firmness | 4.20 | 0.52 | 12.4 | 5.1 | 3.6 | 4.15 | 0.63 | 15.2 | 5.1 | 3.3 | 0.899 | −0.371 | |
| Creaminess | 6.34 | 0.51 | 8.0 | 6.8 | 5.5 | 6.17 | 0.83 | 13.4 | 7.3 | 5.0 | 0.660 | 0.561 | * |
| Salty | 5.41 | 0.45 | 8.3 | 6.2 | 5.0 | 4.91 | 0.44 | 9.1 | 5.5 | 4.4 | 0.078 | 0.568 | * |
| Bitter | 4.27 | 0.45 | 10.6 | 4.9 | 3.7 | 3.61 | 0.36 | 10.0 | 4.0 | 3.0 | **0.019** | 0.485 | |
| Spicy | 3.81 | 0.54 | 14.2 | 4.6 | 3.2 | 3.00 | 0.37 | 12.4 | 3.3 | 2.3 | **0.013** | 0.552 | * |
| Acid | 4.70 | 0.45 | 9.5 | 5.4 | 4.2 | 4.36 | 0.55 | 12.6 | 5.2 | 3.7 | 0.278 | 0.338 | |
| Astringent | 3.99 | 0.44 | 11.2 | 4.8 | 3.7 | 3.52 | 0.30 | 8.4 | 3.9 | 3.2 | 0.058 | 0.445 | |
| Rancid | 2.50 | 0.37 | 15.0 | 2.8 | 1.9 | 2.17 | 0.32 | 14.6 | 2.7 | 1.9 | 0.129 | 0.203 | |
| Intensity | 6.63 | 0.40 | 6.0 | 7.3 | 6.1 | 5.93 | 0.40 | 6.8 | 6.4 | 5.2 | **0.013** | 0.612 | ** |
| Persistence | 6.37 | 0.45 | 7.1 | 6.9 | 5.6 | 5.75 | 0.48 | 8.3 | 6.1 | 4.8 | **0.043** | 0.509 | * |

[1]Black font indicates significance ($p < 0.05$) between batches.
[2]Overall Accep.: Overall acceptance Coef: Pearson correlation coefficient for each sensory descriptive attribute; Sig: Sig (two-tailed) value; *: Sig <0.05; **: Sig <0.01.

the cheese specimens was evaluated by Pearson correlation coefficients (r) and principal component analysis (PCA). Finally, a multivariate linear regression analysis (MLR) was applied to the data using a stepwise regression model to explore the capacity of ultrasonic parameters to predict the variables studied.

## 3. Results and discussion

### 3.1. Ultrasonic parameters

The results obtained for the ultrasonic parameters determined in the cheese batches are presented in Table 2. The ultrasonic longitudinal velocity ($UPV_L$) showed mean values of 1,571 and 1,611 m $s^{-1}$ for the V150-RB and V151-RB transducers, respectively. This ultrasonic parameter is the simplest and most reliable measurement for evaluation of the food properties, including different types of cheese (Benedito, Sanjuan, Carcel, & ; Gallo, Ferrara, & Naviglio, 2018). In general, the values reached in our study were in close agreement with those found in other works for the soft cheese "Torta del Casar" (Crespo et al., 2020) but lower than those found for firm-textured cheese. For Manchego cheese, mean values of ultrasonic velocity ranged from 1,622 to 1,679 m $s^{-1}$ at 8 °C, very similar to those found for fully ripened Cheddar cheese at 12 °C (1,677 m $s^{-1}$) with transducers of 1 MHz and a crystal diameter of 1.9 cm (Benedito et al., 2000a, 2006). In the case of Mahon cheese, a linear relationship between the ultrasonic velocity in cheese cubes and the maturation time was found, attributed to changes in

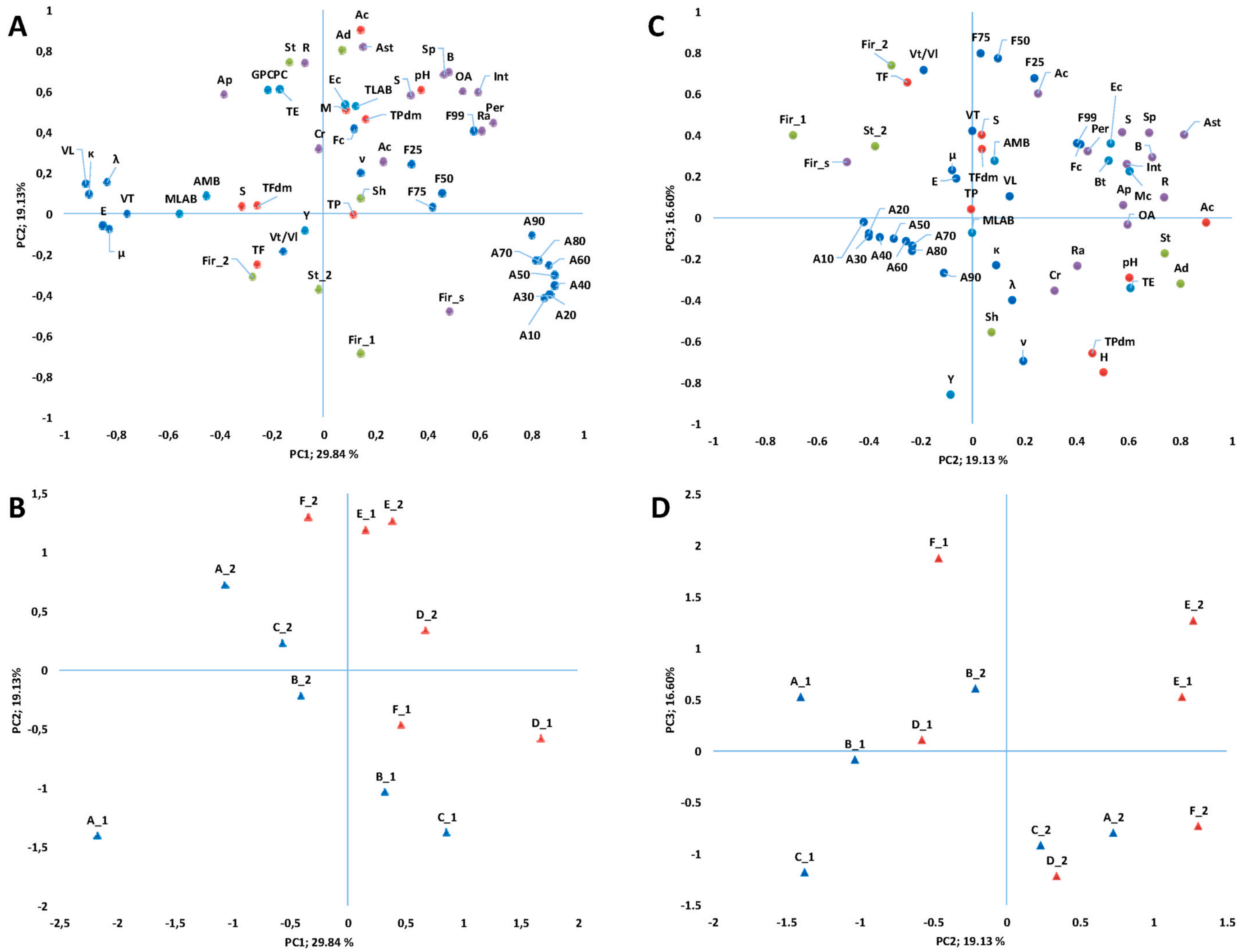


**Fig. 1.** Loading plot (A and C) and score plot (B and D) after principal component analysis of the quality attributes and ultrasound parameters of the soft-bodied cheeses "Torta del Casar" and "Torta de la Serena" in the planes defined by the three first principal components (PC1 and PC2; PC2 and PC3) Microbial parameters (●): TLAB (Bt); GPCPC (Mc); Enterococci (Ec); Enterobacteriaceae (TE); Yeasts (Y). Physico-chemical parameters (●): Moisture (H); Total protein (TP); Total protein DM2 (TPdm); Fat (TF); Fat DM (TFdm); Salt (S) and Acidity (Ac). Rheology parameters (●): TSA Firmness (Fir); TSA Work of Shear (Sh); TSA Stickiness (St); TSA Work of adhesion (Ad); TCA Firmness (Fir2) and TCA Stickiness (St2). Sensorial parameters (●): External appearance (Ra); Internal appearance (Ap); Firmness (Fir_s); Creaminess (Cr); Salty (S); Bitter (B); Spicy (Sp); Acid (Ac); Astringent (Ast); Rancid (R); Intensity (Int); Persistence (Per) and Overall acceptance (OA). "Torta del Casar" (▲); "Torta de la Serena" (▲); winter season (_1); spring season (_2).

textural parameters during ripening (Benedito, Carcel, Clemente, & Mulet, 2000). The ultrasonic shear velocity ($UPV_S$) and the velocity ratio ($UPV_S/UPV_L$) were also determined, and the elastic constants were calculated from the ultrasonic velocity measurements. From these parameters, $UPV_L$ showed the lowest relative standard deviation (RSD; 4.02–6.06%), whereas Lamé's constant ($\lambda$) and the Shear modulus ($\mu$) presented the highest coefficients of variation (17.85%–19.80% and 19.62%–20.71%, respectively). In general, these results are concordant with those obtained by Crespo et al. (2020) for "Torta del Casar" during ripening, the level of dispersion for the parameters being related to the higher velocity obtained with the V150-RB than with the V151-RB transducer. These authors also observed that the %RSD values of the frequency measures were higher for the V151-RB than for the V150-RB transducer, mainly for the highest amplitude frequency, showing 40.28% of the variability in this study. In this sense, it should also be noted that for all the frequency parameters ($FFT_{25}$, $FFT_{50}$, $FFT_{75}$ and $FFT_{99}$), the values obtained with the V151-RB transducer were clearly higher than those obtained with the V150-RB, precisely due to the lower frequency of the latter. Regarding the attenuation-related parameters, slightly higher values were observed in those obtained with the V150-RB transducers, which is logical in the sense that higher values of these attenuation parameters are indicative of a lower attenuation of the ultrasonic waves (Crespo et al., 2020), which must obviously occur with the waves emitted by the lowest frequency transducer, in our study, the V150-RB. In addition, the dispersion data ranged from 17.97% (AT 10 for the V151-RB transducer) to 34.06% (AT 80 for the V150-RB transducer). The high variability found for some ultrasound parameters may be linked to dataset variability in the physico-chemical and texture parameters associated with the sensory quality of cheese.

### 3.2. Microbiological analysis

The counts of AMB and MLAB were higher than 8 log CFU $g^{-1}$ in both batches studied, showing slightly higher mean values of MLAB counts ($p = 0.067$) in the Serena batch than in the Casar batch (Table 3). MLAB are reported to be the predominant microbial groups during the ripening of soft ewe's milk cheeses clotted with vegetable rennets, such as "Serra da Estrela", "Serpa", "Queso de la Serena" and "Torta del Casar" cheese (Del

**Table 5**
Statistical indicators, MLR coefficients and their significance for the developed MLR models for the TCA parameter firmness and sensorial attribute using a stepwise regression technique.

| | Response variables | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Firmness (TCA) | | | | Sensorial attribute “Intensity” | | | |
| Model Summary | | | | | | | | |
| R | 0.928 | | | | 0.918 | | | |
| R-squared | 0.861 | | | | 0.843 | | | |
| R-sq (adj)[a] | 0.809 | | | | 0.785 | | | |
| Std error | 1130 | | | | 0.24 | | | |
| ANOVA | | | | | | | | |
| F-values | 16.5 | | | | 14.4 | | | |
| *P* values | 0.001 | | | | 0.001 | | | |
| Coefficients | | Explanatory variables | | | | Explanatory variables | | |
| Term | (Intercept) | $F_{75}$ | $F_{99}$ | AT 60 | (Intercept) | $F_c$ | VL | $F_{99}$ |
| *Unstd Coeff.*[b] | | | | | | | | |
| B | −30198 | 0.18 | −0.04 | −1584 | 7.550 | 3.7E-06 | −3.0E-03 | 4.3E-06 |
| Std. Error | 6452 | 0.029 | 0.012 | 639 | 2.476 | 7.7E-07 | 1.2E-03 | 1.8E-06 |
| *Std Coeff.*[c] | | | | | | | | |
| Beta | | 1.200 | −0.686 | −0.373 | | 0.707 | −0.363 | 0.361 |
| t-value | −4.681 | 6.26 | −3.283 | −2.479 | 3.049 | 5.035 | −2.380 | 2.369 |
| *P* value | 0.002 | 0.000 | 0.011 | 0.038 | 0.016 | 0.001 | 0.045 | 0.045 |
| VIF[d] | | 2.115 | 2.519 | 1.300 | | 1.008 | 1.190 | 1.185 |

[a] R-sq (adj): Adjusted R-squared.
[b] Unstd Coeff.: Unstandardized coefficients.
[c] Std Coeff.: Standardized coefficients.
[d] VIF: Variance inflation factor.

Pozo, Gaya, Medina, Rodríguez-Marín, & Nuñez, 1988; Gonçalves et al., 2018; Ordiales, Benito, et al., 2013; Tavaria & Malcata, 1998), in which they commonly reach viable numbers of 8 log CFU $g^{-1}$ by the time of consumption. The rest of the bacterial groups studied ranged from 4.9 log CFU $g^{-1}$ of GPCPC in the Serena batch to 6.4 log CFU $g^{-1}$ of enterococci in the Casar batch. This last microbial group seemed to present ($p < 0.1$) higher counts for the Casar batch, while TLAB, GPCPC and Enterobacteriaceae did not present significant differences. These microbial populations are considered typical of chesses made from raw milk (Freitas & Malcata, 2000). Enterobacteriaceae showed the greatest variability in the Casar batch with a %RSD value of 28.5%, in contrast to AMB and MLAB with %RSD values lower than 5% for both batches studied.

Regarding fungal counts, the mean values of the yeast counts were higher than 3 log CFU $g^{-1}$, in agreement with the results reported in other works (Ordiales, Martín, et al., 2013), with higher mean values ($p = 0.068$) observed in the Serena batch. In the case of mould, the counts found in the paste of the cheeses were unremarkable (Table 3).

### 3.3. Physicochemical parameters

The values of physicochemical parameters for the cheese batches are presented in Table 3. There were no statistically significant differences ($p > 0.05$) in the moisture, total protein, fat and salt concentration (calculated from chloride concentration) between the two batches studied, with ranges of 45.8%–46.7%, 21.1%–21.4%, 29.1%–29.4% and 2.1%–2.3%, respectively. In general, these results were similar to those reported for other soft cheeses, including “Torta del Casar” (Ordiales et al., 2014; Roa, López, & Mendiola, 1999; Sanjuán et al., 2002). However, significant differences were found for pH values between the batches, probably reflecting the metabolic activity of MLAB in this type of raw ewe’s milk cheese during ripening. In fact, the lower pH value in the Serena batch may be associated with the higher MLAB count found in this batch. Salt concentration and acidity showed the highest variability in physicochemical parameters, with %RSD ranges of 33.4%–43.6% and 39.6%–39.8%, respectively. On the contrary, pH and total protein presented %RDS values equal to or less than 5%.

### 3.4. Texture analysis

Table 3 also shows the descriptive statistics of TCA and TSA parameters in the two batches studied. For TCA, the mean values of firmness and stickiness ranged from 3412 to 3796 g and from −39.8 to −28.7 g, respectively; the absence of differences between the two type of cheeses was mainly due to the great intra-batch variability found in these parameters (%RDS higher than 65). The mean values of the TCA parameters are similar to those observed for other cardoon-coagulated cheeses with extensive proteolysis (Crespo et al., 2020). In the same way, the results for TSA parameters did not show any differences among the studied batches, and the observed value ranges were comparable to those previously reported for this type of cheese (Delgado, Rodríguez-Pinilla, González-Crespo, Ramírez, & Roa, 2010). Again, the intra-batch heterogeneity was obvious, the variability of TSA parameters clearly higher for the Casar batch with %RSD values over 65%, whereas for the Serena batch, the values reached were lower than 52%. This highlights the need to non-destructive assessment of these essential parameters for the quality of this type of cheese.

### 3.5. Sensory analysis

The results for the sensory descriptive attributes of the cheeses and their correlation with overall acceptance are shown in Table 4. In terms of variability, the descriptors showed similar %RDS values for both batches studied except for acceptance descriptors, which showed a higher dispersion in the Serena batch (data not shown). The values of the attributes rind appearance, bitter, spicy, intensity and persistence were higher for the Casar batch. Except for the descriptor “bitter”, all of them were correlated with overall acceptance together with creaminess. The relation between creaminess and overall acceptance in the sensory analysis has been broadly described for soft-bodied cheese associated with not only the proteolytic activity of the vegetable rennet but also the microbial population (Guiné, Tenreiro, Correia; Tavaria & Malcata, 2003). A high enzymatic activity in this type of cheese has also been related to such descriptors as bitter, spicy, intensity and persistence (Ordiales et al., 2014). However, with respect to the descriptor firmness, differences found between both batches were not significant ($p < 0.05$),

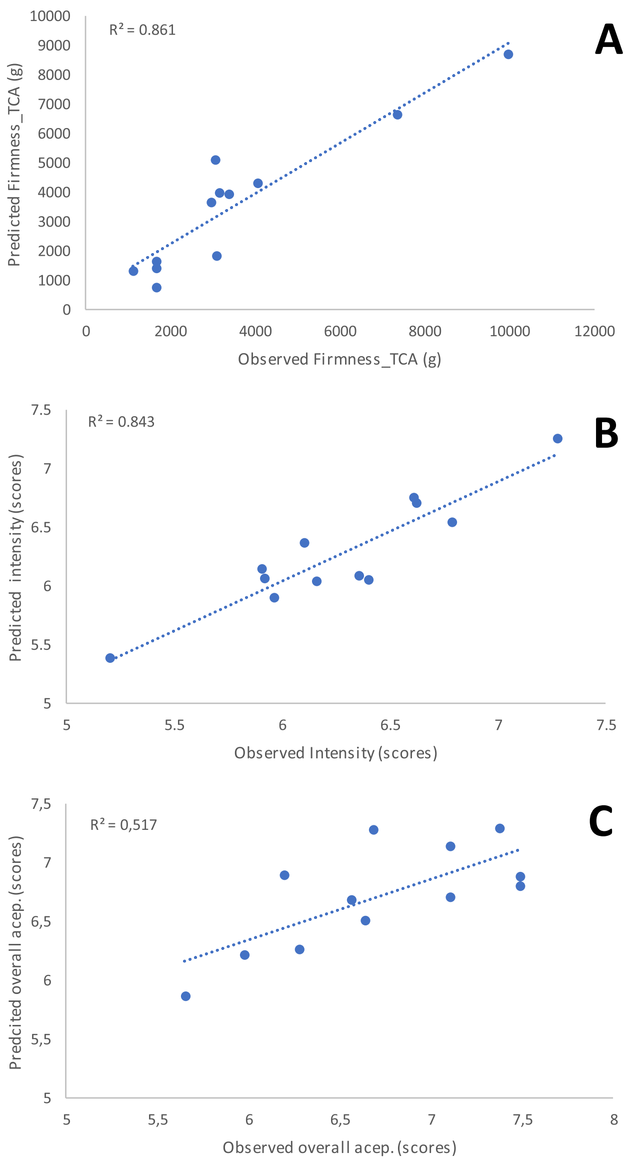


**Fig. 2.** Comparison between the observed and predicted values of firmness (TCA) (A) and the sensorial attribute "intensity" (B) for multiple linear regression (MLR) and overall acceptance (C) for simple linear regression (SLR).

and its variability in this study (%RDS 12.4–15.2) was not significantly correlated with overall acceptance.

### 3.6. *Relationship of microbial, physico-chemical, textural and sensory parameters with ultrasonic parameters*

Correlations of the ultrasound parameters obtained using both transducers (V150-RB and V151-RB) with microbial, physico-chemical, textural and sensory parameters evidenced that the V150-RB transducer performed better than the V151-RB transducer (Table S1). Thus, ultrasonic parameters obtained with the V150-RB transducer were used for principal component analysis (PCA) (Fig. 1).

The PCA of the microbial, physico-chemical, textural, sensory and ultrasonic parameters showed major differences among the cheese samples manufactured in different PDO, allowing their discrimination (Fig. 1B). PC1 accounted for 29.84% of the variance and was mainly defined by attenuation in the positive axis and most of the velocity-related parameters along the negative axis; however, it was also defined by several sensory parameters and, to a lesser extent, by microbial counts (MLAB and AMB) (Fig. 1A). Concretely, the descriptors firmness (Fir_s) and persistence (Per) showed the most significant negative correlation with $\kappa$ (R = −671 and −689, respectively), whereas for rind appearance (Ra), the highest absolute value of the correlation coefficient was with $\mu$ (R = −734) and $E$ (R = −715). The common terms "firmness", "rigidity" and "stiffness" can be related to $\kappa$, $\mu$ and $E$, respectively (Ludger & Teixeira, 2007). The shear modulus ($\mu$) is concerned with the deformation of the cheese when a force is applied parallel to its surface; therefore, cheeses with a rigid and well-formed rind would be shown lower values for this ultrasonic parameter.

The PC2 and PC3 explained 19.13% and 16.60% of the variance, respectively (Fig. 1C), being able to observe a certain distribution of samples according to the season produced (Fig. 1D). PC2 was defined by the TSA texture parameters (stickiness and work of adhesion by the positive axis; firmness by the negative axis), fat acidity index, pH, sensorial descriptors (rancid, astringent, spicy and bitter) and to a lesser extent by microbial counts (GPCPC and enterobacteria) (Fig. 1C). The contribution to the lipolytic and proteolytic activities of these microbial groups can explain their relation to the abovementioned parameters. The relationship between textural and proteolytic activity has been described for this type of cheese, observing that the degradation of the casein matrix decreases the hardness and consistency of "Torta del Casar" and increases their adhesiveness during ripening (Delgado et al., 2010). High pH values in "Torta del Casar" have been associated with the buffering effect generated by the products of protein hydrolysis (Ordiales et al., 2014), which also contribute to bitter peptide accumulation in cheese (Broadbent, Strickland, Weimer, Johnson, & Steele, 1998). In addition, the microbial lipolytic activity in cheeses yielded free fatty acids, some of them associated with spicy and rancidity descriptors (Salles et al., 2002). Several sensory parameters, including some of those mentioned above, presented the highest correlation coefficients (>0.683) with the frequency parameter $FFT_{99}$ and HAF, such as the descriptors salty, spicy, fat acidity index, intensity and overall acceptance (Fig. 1C). Therefore, structural changes in the cheeses associated with higher microbial proteolytic and lipolytic activities may explain these correlations. With respect to PC3, it was defined by other frequency parameters ($FFT_{25}$, $FFT_{50}$ and $FFT_{75}$) and $UPV_S/UPV_L$ in the positive axis and mainly by $\nu$ in the negative axis (Fig. 1C). This last ultrasound parameter showed the highest positive values for the correlation coefficient (R = 0.639) with total protein DM, whereas $FFT_{75}$ presented a significant correlation (R = 0.604) for the texture TCA parameter firmness. The latter result would suggest that stiffer samples would favour the transmission of higher-frequency ultrasonic waves. The rest of the physico-chemical parameters were not clearly associated with any PC, and their explained variability was distributed over the three PCs studied.

In order to predict the behaviour of the microbial, physico-chemical, textural and sensory parameters of the cheeses by the ultrasonic parameters as predictor variables, the multivariate linear regression method (MLR) was employed (Table 5). The predictive models for the TCA parameter firmness and sensory descriptor intensity showed the highest correlation coefficients (r = 0.928 and 0.918, respectively), indicating that 80.9% and 78.5% of the variation in these relevant variables for the quality of cheeses could be predicted from several secondary ultrasound parameters, according to the adjusted determination coefficient (adj-$R^2$) of the MLR models (Table 5). The explanatory variables to predict the outcome of TCA firmness were $FFT_{75}$, $FFT_{99}$ and AT60, whereas in the case of the sensory descriptor intensity they were HAF, VL and $FFT_{99}$, showing no collinearity between variables for both models (VIF ≥10). This last ultrasound parameter also provided the best adjusted simple linear regression model to the values of overall acceptance. Based on the regression models achieved, the relationship between ultrasound parameters and TCA firmness, sensory descriptor intensity and overall acceptance is shown in Fig. 2. Therefore, in order to interpret these results, the role of the hitherto secondary ultrasound parameters in cheese quality control, such as FFT and attenuation, should be recalled. The attenuation values were already shown to be a useful tool for discriminating soft cheeses with different defects (Crespo et al., 2020), whereas FFT and attenuation showed the greatest significance in their linear correlations with parameters of texture for cooked pork loin (González-Mohino et al., 2021).

## 4. Conclusion

In this study, we discussed the usefulness of ultrasound parameters in the quality control of soft cheeses, confirming the high correlation between several ultrasonic parameters and changes in the physicochemical, textural and sensorial properties of this product, which are partially associated with microbial activity. To the best of our knowledge, in soft cheeses, such as "Torta del Casar" and "Queso de la Serena", this is the first literature study to report the predictive potential of several secondary ultrasonic parameters for relevant quality attributes of cheeses and, in turn, to validate the on-line capability of ultrasound for non-destructive evaluation of soft-cheese quality at an industrial level.

## CRediT authorship contribution statement

**Abel Crespo:** Term, Conceptualization, Validation, Formal analysis, Investigation, Data curation, Writing – review & editing. **Antonio Jiménez:** Term, Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – review & editing, Supervision, Funding acquisition. **Santiago Ruiz-Moyano:** Term, Conceptualization, Validation, Investigation, Resources, Writing – original draft, Supervision, Project administration, Funding acquisition. **Almudena V. Merchán:** Methodology, Investigation. **Ana Isabel Galván:** Methodology, Investigation. **María José Benito:** Benito, Methodology, Writing – review & editing. **Alberto Martín:** Term, Conceptualization, Methodology, Validation, Formal analysis, Data curation, Writing – review & editing, Supervision.

## Declarations of competing interest

None.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2021.108405.

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